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ADJUSTMENTS OF NERVE ENDINGS*

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The nerve cell is the structural unit which underlies nervous activity. Nerve cells function in groups and in chains, the separate cells being linked with one another at synapses through their nerve fiber endings. Nerve endings are also the structures which receive impulses from the sensory apparatus and deliver impulses to the motor apparatus. It is obvious, therefore, that changes and adjustments in nerve endings may be of profound importance.

In his classic work Cajal[1] has presented a very complete picture of microscopic changes in nerve cells and endings as these undergo degeneration and regeneration in various parts of the peripheral and central nervous system. His observations were made on fixed and stained material. Such a technique did not permit observations of the sequence of changes in the same individual endings. Nevertheless, Cajal had no difficulty in recognizing the normal resting nerve ending, the ending with a growth cone at the tip, and the retracting ending with a retrac-

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tion club at the tip. He also recognized that nervous autotomy sometimes occurred.

After his invention of the tissue culture method Harrison[2] made the first important direct observations on the living young nerve fibers as these grew out from explanted nerve cells. He described in detail the activities of the growth cones and the part they play in the spinning of new nerve fibers. Many other investigators have since used the tissue culture method in studying nerve cells explanted from various parts of the nervous system.

An interesting and significant point is that so far in tissue culture the myelin sheath has not been differentiated. This is true even in cultures in which nerve cells and neurilemma cells are kept in intimate association with one another. The nerve endings that are present in tissue cultures, therefore, can hardly be regarded as mature endings of the type that in the living animal represent the terminations of myelinated fibers.

Some years ago I found that it was possible to watch individual nerve fibers for prolonged periods in living frog tadpoles. A simple technique was used involving microscopic examination of the animal in a special upright chamber, the animal being temporarily immobilized with a weak anesthetic. With care the entire history of the same nerve fiber could be obtained for periods of several days or weeks, the animal being kept in good condition throughout the observations. Moreover, it was found possible to take satisfactory ciné-photomicrographs revealing minute details of the changes in nerve fibers and their endings. Especially valuable were pictures of the "fast motion" type which vividly revealed relatively slow changes.

The growth of individual nerve sprouts, the movements of neurilemma cells, and the process of myelin sheath formation were first studied.[3,a,b,c] This was followed by experimental studies of the phenomena accompanying nerve irritation and recovery, injury and repair,[3,d] and by studies of the effects on nerve fibers of alcoholic intoxication[3,e] and metrazol treatment.[3,f] Recent work, not yet published, has dealt with the effects on nerve fibers and their endings of electric shocks and of insulin treatments. The increasing use of shock therapy in the treatment of human mental disorders makes this field of investigation of timely interest. Other recent work, also as yet unpublished, has dealt with the changes in nerve endings during periods of normal rapid growth in size of the animal, during alternating periods of starvation and good nutrition, during alternating periods of exposure to strong and weak anesthetics, and during temperature variations.

In this lecture I propose to give an account of the fundamental structural changes in nerve endings that are discernible under both normal and experimental conditions. This account includes changes associated with growth, regeneration, starvation, treatments with chloretone, alcohol, metrazol, insulin, electricity, hypertonic salt solutions, X-rays, and temperature variations. This account also includes adjustments of nerve endings associated with local tissue changes, such as occur during the progress of myelination, during tissue regulation after wound infliction, during the establishment of collateral innervation after experimental denervation of nearby territory, and during various local cellular movements.

Observations and Experiments

A typical mature tree of endings of a myelinated fiber is shown in the accompanying illustration (Fig. 1).* This sketch gives the appearance not only of the ordinary endings in the resting stage but also of those in stages of growth, retraction, irritation, and degeneration.

Extension and Retraction of Nerve Endings During Early Growth and Regeneration

The phenomena of marked extension and retraction are most readily to be seen at the tips of young or rapidly regenerating nerve fibers. In the living frog tadpole at an early stage of development cutaneous nerve fibers may be watched as they grow out toward the skin (Fig. 2). Typical mobile growth cones are present at the tips. These advance in somewhat sporadic fashion. The rate of progression, therefore, is subject to great variation. About 40 micra per hour represents rather rapid growth. At the tips of the proximal stumps of regenerating nerve fibers growth cones also develop and move out in similar fashion.

Second and later growth cones of other nerve fibers follow the line laid down by the first and a small nerve is thus formed. The fibers are naked at first. Soon, however, neurilemma cells move out along the nerve, multiply, and play an important role in the formation of both myelin sheath and neurilemma. Characteristic terminal arborizations, such as are shown in Fig. 1, ultimately develop on myelinated fibers,

* All figures except the last (Fig. 18) represent nerve fibers in the tail of the frog tadpole.

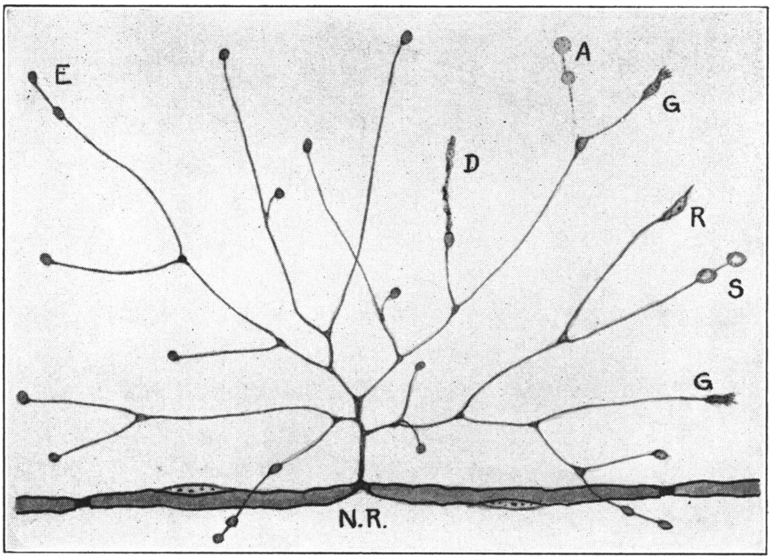

Fig. 1—Diagram showing a cluster of cutaneous nerve endings. At a node of Ranvier (N.R.) a collateral branch is given off which subdivides as shown. At the tips of most of the branches are typical resting endings (E). Two endings are characterized by growth cones (G), one by a retraction club (R), and one by a markedly swollen condition (S). A short length of one branch is in degeneration (D) and another in process of autotomy (A). This illustration of frog tadpole nerve endings peripherally located affords an interesting comparison with that of cat nerve endings centrally located (cf. Fig. 18).

not only at the end of the most distal myelin segment, but also at collateral side branches which appear at some of the nodes of Ranvier. At the tip of each branch of a terminal arborization is a small spherical or ovoid end bulb (bouton). Any one of these, however, may become transformed into a growth cone and advance, or into a retraction club and retreat.

Rapidly growing nerve endings under certain conditions may cease their advance and then undergo rapid retraction. This sometimes takes place spontaneously. It may also be induced readily by various experimental procedures. Among the methods which I have used to bring about retraction of growth cones are the following: treatment with alcohol, chloretone, metrazol, insulin, electricity, heat, hypertonic sodium chloride solution, starvation, and wound infliction by cutting or bruising nearby tissues with resulting general tissue adjustments.

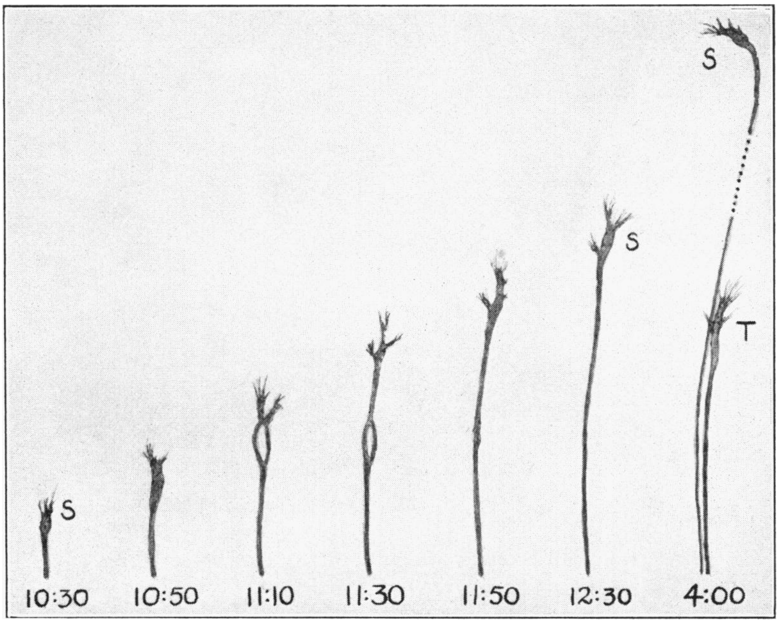

Fig. 2—Normal rapidly growing nerve endings in a young tadpole shortly after hatching. A pioneer growth cone (S) at the tip of a single nerve fiber advanced through the tissues of the tail fin, its progress being shown from 10:30 A.M. to 4:00 P.M. At the end of one hour it had traveled about 45 micra; at the end of two hours 85 micra; and at the end of five and one-half hours 220 micra. (The dotted line in the final sketch indicates that a part of the fiber has been omitted from the drawing.) A second growing tip (T) followed the line laid down by the first.

Retraction is initiated first by the transformation of a growth cone into a retraction club, and then by a regressive flow of neuroplasm proximally (Fig. 3). A fine pointed axial filament is often left for a time; then this is withdrawn. Sometimes a vigorous production of transient knob-like excrescences accompanies the retraction. These resemble similar excrescences that often characterize cells dividing by mitosis.

The speed of retraction varies greatly. Over a brief period the amount of retraction sometimes exceeds the rate of one micron per minute, as in the case illustrated (Fig. 3). Usually, however, the rate of retraction is not so great.

It is possible to induce conditions experimentally which alternately favor growth and retraction. The example given (Fig. 4) shows how variations in the degree of chloretone anesthesia are effective in bringing about conditions which favor alternately retraction and growth.

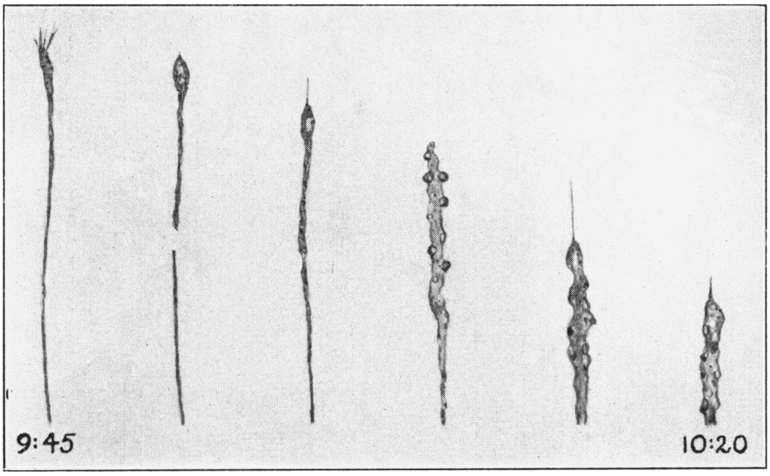

Fig. 3—Rapid retraction of the tip of a regenerating nerve fiber. An active growth cone advancing through the tissues at 9:45 A.M. became transformed into a retraction club and then vigorously retracted. At 10:20 A.M. it was in the position shown, having retracted about 45 micra.

In an earlier investigation[3,f] I have described similar instances in metrazol-treated tadpoles.

As an ending recovers and grows out again after a period of retraction, it may, or may not, follow its former course. Case histories which illustrate each of these possibilities have been presented in a previous paper which described nerve ending reactions in tadpoles subjected to alcoholic intoxication.[3,e]

Changes in the Endings of the Terminal Arborization of Myelinated Fibers During Normal Growth

In general, as a tadpole grows in size the clusters of cutaneous nerve endings of myelinated fibers also grow. Although the majority of the endings of a cluster are directed superficially toward the skin, a few may be directed deeply. Aberrant deep sprouts of this type which are of no service as cutaneous endings undergo readjustment along one of the following lines: they may advance further through the tissues and then extend superficially to establish cutaneous connections; they may retract variable distances and then change their direction of growth to reach the skin; they may suffer a variable amount of degeneration or autotomy with subsequent growth to a superficial position; or, finally,

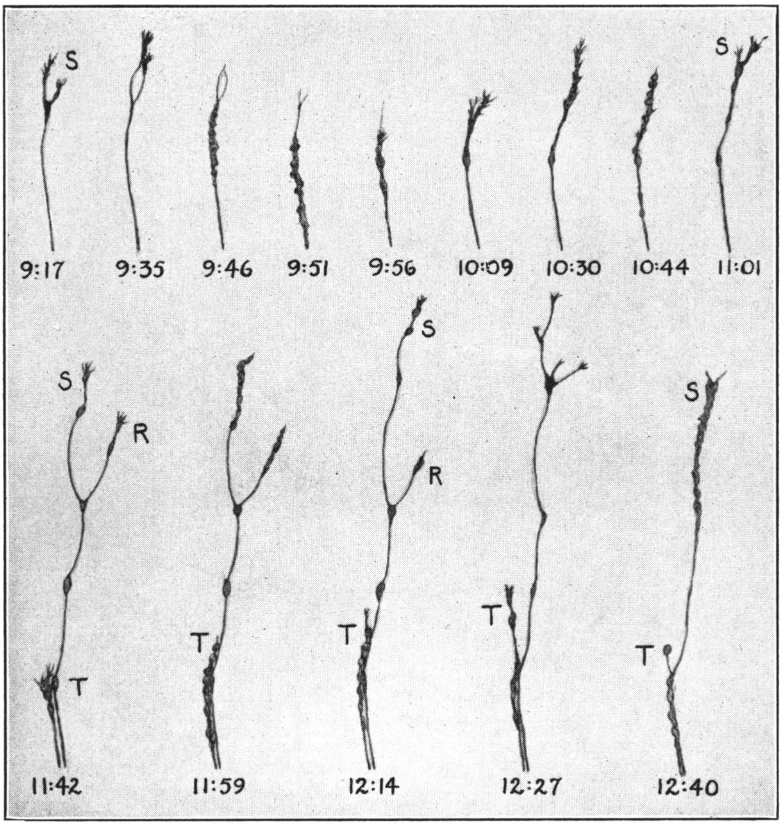

Fig. 4—Successive retraction and extension of regenerating nerve endings, correlated with treatments with strong and weak chloretone solutions. An active pioneer growth cone (S) and a second growth cone (T) were watched in a tadpole under chloretone anesthesia from 9:17 A.M. to 12:40 P.M. Strong chloretone treatments were given from 9:37 to 9:52, from 10:33 to 10:53, from 11:49 to 12:07, and from 12:29 to 12:40. At other times over the period illustrated the tadpole was immersed in either pond water or very weak chloretone solution. Typical retraction stages followed each of the four strong chloretone treatments; as at 9:46 and 9:51, at 10:44, at 11:59, and at 12:40. Typical growth stages characterized the other periods while the tadpole recovered in pond water or in weak chloretone. Before 11:42 a second growth cone (T) grew into the field illustrated, following the line of the pioneer fiber. A short branch (R) was present for about one hour, but retracted fully before 12:27.

they may be completely eliminated by either full retraction or autotomy.

I have obtained several complete case histories of the changes in entire trees of nerve endings while the frog tadpole grew markedly in size over a period of a month.* The example given here illustrates the

* Details of these have now been published in the *J. Comp. Neurol.*, 1942, *76*:57.

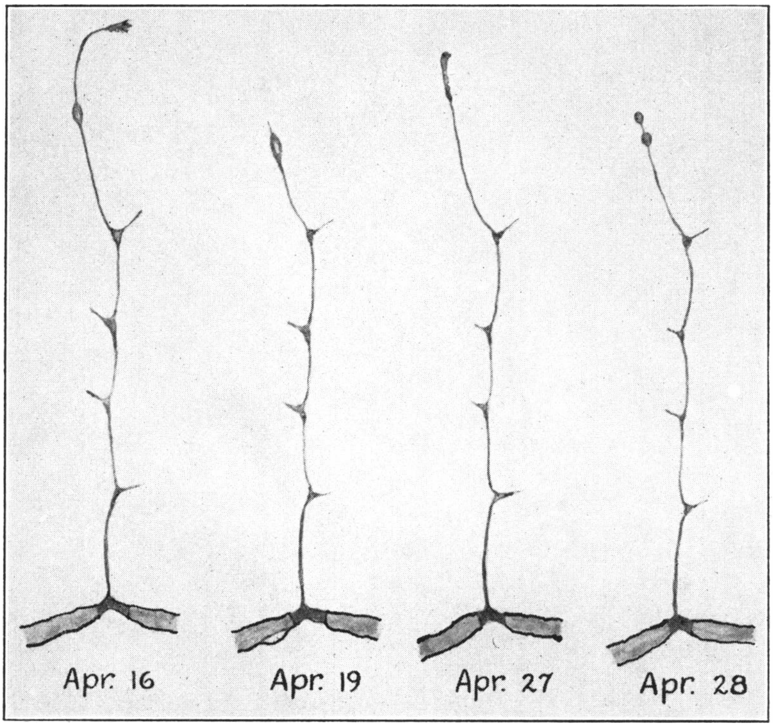

Fig. 6—Successive retraction and extension of a nerve ending correlated with successive periods of starvation and good nutrition. A motion picture record of this case was obtained. On April 16th a nerve ending with growth cone tip was observed slowly advancing. After three days of starvation definite retraction had taken place. On April 19th a typical retraction club was present at the tip. From April 20th to 27th food was made available and the ending again advanced. A second period of starvation was followed by a second retraction on April 28th.

changes of only two of the endings of a terminal arborization (Fig. 5). These are sufficient, however, to indicate that extension, retraction, branching, and elimination of branches all may take place during normal growth. They indicate further that a gradual increase in nerve ending extent is correlated with the increase in the growing terrain, and that a marked decrease in nerve ending length finally results when the tail fin undergoes reduction in size as metamorphosis starts.

Nerve endings often reflect general growth conditions. They are markedly influenced by variations in nutrition. Thus, in two tadpoles in which end arborizations were under observation for several weeks it was noted that a complete change of aquarium water and food mate-

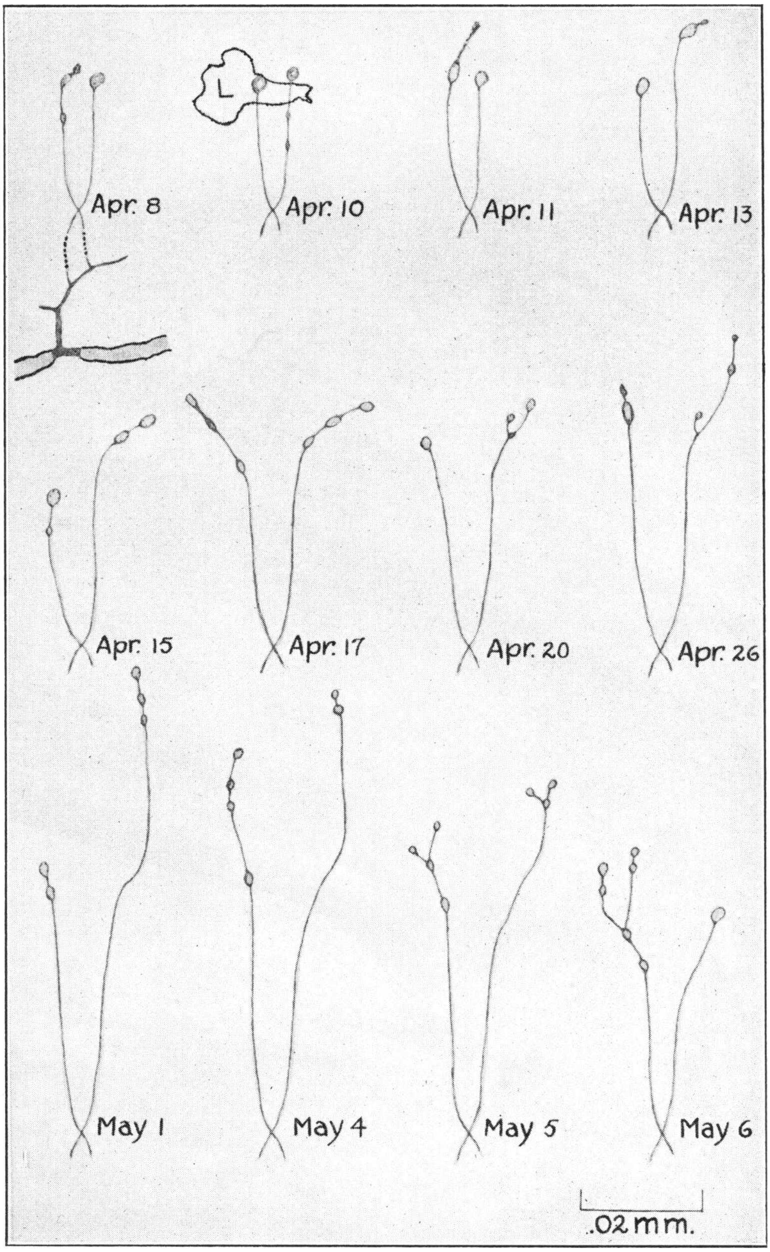

Fig. 5—Adjustments of two nerve endings over a period of four weeks during marked growth of the tadpole. The sketches are made exactly to scale from motion picture records. A distance of 0.02 mm. (20 micra) is indicated below. During the period from April 8th to May 6th, the ending exhibited extension, retraction, and branching. On April 10th a leukocyte (L) approached and pressed against the ending at the left. Extension of this ending occurred shortly afterward. The final reduction in length of the endings from May 4th to May 6th was correlated with reduction in the size of the tail fin, as the tadpole began metamorphosis. (The dotted lines in the first sketch indicate that some of the nerve fiber length has been omitted from the drawing.)

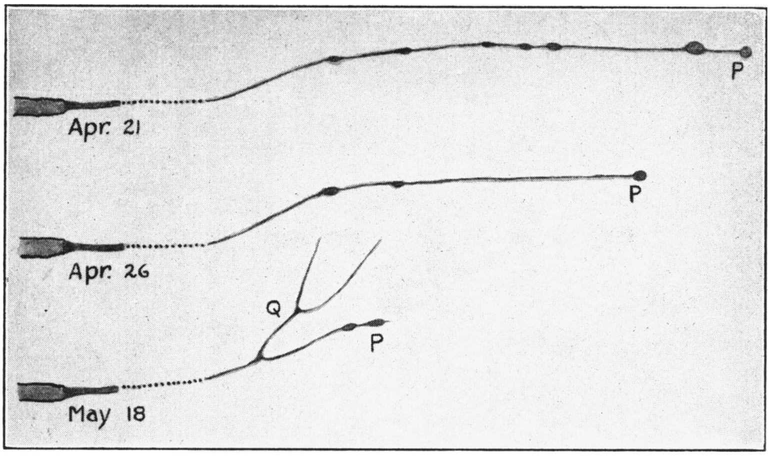

Fig. 7—Nerve ending retraction and new branch formation correlated with starvation succeeded by good nutrition. A motion picture record of this case was obtained. From April 18th to April 26th a tadpole was deprived of food; thereafter food was made available. A nerve ending (P) retracted about 18 micra during the period from April 21st to 26th. After that, in spite of the fact that food was made available, retraction continued to the extent of about 60 micra. Proximally along the retracting ending, however, a new branch (Q) grew out. (The dotted line indicates that some of the length of the fiber has been omitted from the drawing.)

rials brought about a spurt of general bodily growth within a few days. In each of these animals the nerve endings under observation exhibited, likewise, marked growth during the same period. Conversely, if food conditions are not quite favorable enough to support general bodily growth in a young tadpole, nerve endings are likely to exhibit little or no growth. Individual branches of a terminal arborization may react independently. One ending may grow while another retracts.

Terminal arborizations of regenerating fibers develop in regenerating zones about two weeks after partial tail amputation. Myelination progresses rapidly at this time. Case histories of the changes in the nerve endings of such regenerating regions are quite similar to those of normal growth, although somewhat greater variation is displayed.

Effects of Starvation on Nerve Endings

Starvation causes general changes in the tissues of the tadpole's tail. Epithelium, muscle, connective tissue, and nerve all exhibit definite irritative changes. Nerve endings may undergo swelling, retraction, autotomy, and degeneration. Examples of each of these have been observed.

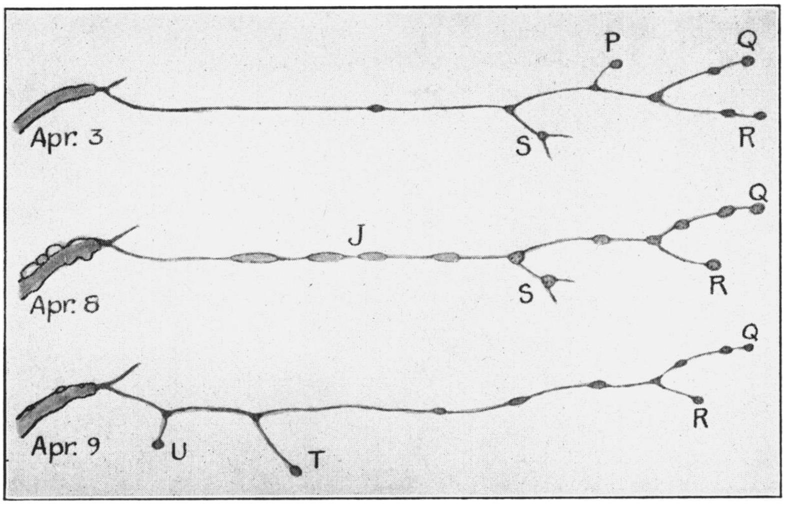

Fig. 8—Some changes in nerve endings correlated with alcoholic intoxication. Tadpole subjected to 3% alcohol for 47 minutes on April 3rd and again for 95 minutes on April 8th. Nerve ending, P, was eliminated after the first treatment. Ending, S (with its two sub-branches which are not fully shown in the drawing), was eliminated after the second treatment. Ending, R, underwent some retraction. New branches, T and U, appeared after the second treatment. Marked uneven swelling of the fiber also was visible in the region of J, on April 8th; also at Q, R, and S.

Young tadpoles are particularly responsive to alternating periods of starvation and good nutrition. Their nerve endings display regressive changes alternating with progressive changes. In the example given (Fig. 6) successive advance and retreat of a nerve ending were induced. This was correlated with the general nutritive conditions. While exact correlation was not found for all individual nerve endings in cases of this type, it was quite obvious that starvation brought about general regressive changes in both nerve fibers and their endings.

The other case cited (Fig. 7) indicates that regressive change in an ending initiated by starvation may continue, as regards that particular ending, even after good nutrition is restored. The recovery in this case was shown by the development of a new branch some distance proximally to the retracting ending.

A nerve ending in another starved tadpole formed the subject of an interesting motion picture record. As the picture was being taken, a greatly swollen end bulb underwent autotomy, after which it was ingested by a phagocytic leukocyte, while a new abortive growth cone appeared at the new tip of the nerve ending.

Some Changes in Nerve Endings Correlated with Alcoholic Intoxication

Frog tadpoles, immersed in weak solutions of alcohol for suitable periods of time, become slightly dazed. Their nerve fibers and nerve endings exhibit conspicuous changes. These changes have been described elsewhere in some detail with illustrations of many individual case histories.[3,e] Myelinated fibers exhibit vacuolation, fibrillation of the axis cylinder, swelling, progressive separation of axis cylinder and myelin sheath, globule formation, and in extreme cases complete degeneration of some myelin segments with or without degeneration of the corresponding length of axis cylinder.

The cutaneous nerve endings exhibit swelling, retraction, and degeneration with or without autotomy. Sometimes retraction is sufficient to bring about elimination of a branch. During the recovery period following alcoholic intoxication, irritated endings may exhibit reduction of swelling, extension, and the formation of new branches. Endings of the fiber illustrated (Fig. 8) exhibit swelling, retraction, elimination of some branches, and formation of other new ones. A normal end bulb is in the gel state. With increasing irritation an end bulb changes progressively from the gel state toward the sol state; with recovery it returns to the gel state.

The effects of alcohol on growing nerve sprouts during rapid regeneration are even more striking. I have taken motion pictures of growth cones as they are transformed into retraction clubs which then rapidly retreat. With restoration of normal conditions the retracting nerve tips within a short time develop new growth cones and resume their advance through the tissues. They may proceed along their former course or along a new path. Examples of each have been observed and recorded ciné-photomicrographically. Rapidly regenerating nerve tips exhibit in somewhat exaggerated fashion the same fundamental changes shown by the resting nerve endings of terminal arborizations.

The Neurilemma in Relation to the Duplication of an Original Nerve Pattern by a Regenerating Fiber

An important question in the regeneration of a nerve fiber and its endings concerns the extent to which exact duplication of an original pattern may be expected. Several case histories of experimental nerve

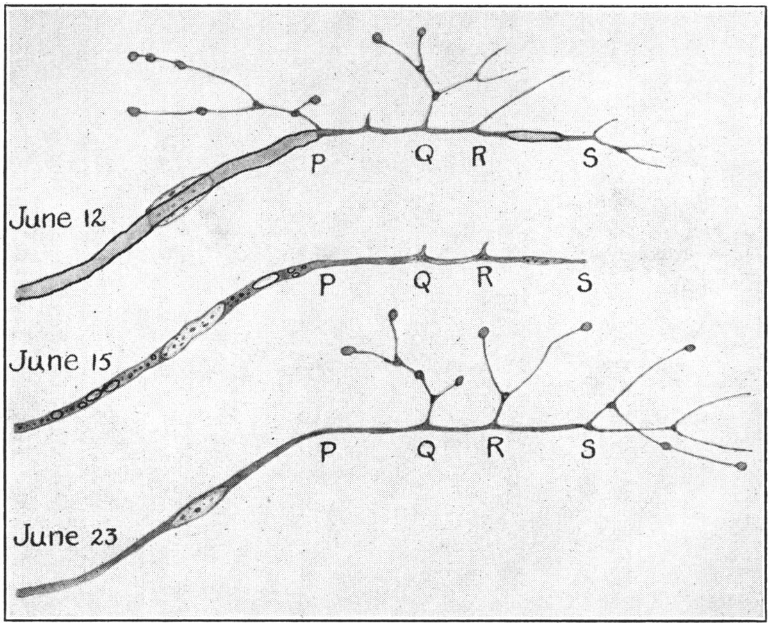

Fig. 9—The neurilemma as a directing factor during nerve regeneration in the exact duplication of an original pattern. On June 12th a nerve fiber ended as shown with branches at P, Q, R, and S. The fiber was then sectioned some distance proximally. On June 15th degenerating remnants of the fiber were still visible within the neurilemma. The neurilemma was visible as far distally as S with short funnel-like extensions at Q and R, though not at P. On June 23rd the regenerating fiber had grown back into the old neurilemma and had given rise to a set of endings at Q, R, and S. Exact duplication of the original nerve pattern corresponded only to the part enclosed by neurilemma. The pattern of branching of the fiber not ensheathed by neurilemma was unlike the original pattern. Collateral branches appeared at Q and R, but not at P, a result possibly correlated with the presence or absence of neurilemma side extensions at these points (cf. also Fig. 10).

section and of spontaneous degeneration and regeneration of fibers under observation have yielded clear-cut data on this point. The example given (Fig. 9) shows that a regenerating fiber growing within the old neurilemma tube duplicated the original pattern only as far as the neurilemma extended. Any branches that arose distally to this were naked and their pattern of branching bore no special resemblance to the original pattern.

Other case histories have given similar results. The inference is clear that the patterns of free unsheathed nerve endings which suffer degeneration are not exactly reproduced by regenerative processes. This fea-

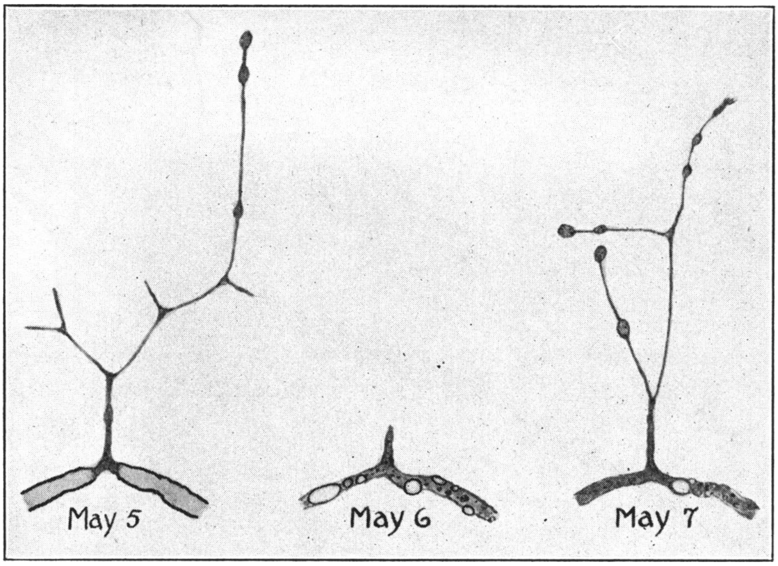

Fig. 10—Severe injury of a nerve fiber by metrazol treatment, with degeneration of a collateral branch with its cluster of endings followed by regeneration of a new collateral with a new pattern of endings. A motion picture record of this case was obtained. On May 5th a collateral of a myelinated fiber at a node of Ranvier branched as indicated. (The drawing shows the tip of only one of the branches.) Metrazol was administered in strength sufficient to induce degeneration of the entire collateral branch, as well as a short length of the main myelinated fiber. On May 6th the neurilemma sheath was present filled with degenerative debris of the fiber. On May 7th the main fiber had regenerated and given rise to a collateral at the former node of Ranvier site. Three endings developed on the collateral. The pattern of branching, however, was quite unlike that of the original pattern before the injury.

ture of nerve ending regeneration is of decided significance in connection with the question of synapse stability in the central nervous system, a problem to be discussed below in this paper.

Degeneration and Regeneration of Nerve Endings in Metrazol-treated Tadpoles

Profound alterations become visible in the tissues of metrazol-treated tadpoles. The circulation is markedly affected. It may stop temporarily in the small vessels near the edge of the tail fin. Muscle, epithelium, and nerve all exhibit structural changes.

The nerve changes are of special interest. They afford a basis for the interpretation of certain results that have been obtained in the treatment of human mental disorders by injections of metrazol. In a previous paper[3,f] I have described the results of subjecting tadpoles to mild, moderate and severe treatments with metrazol. Case histories have been presented which illustrate nerve fiber recovery after various gradations of irritation and injury. One example is offered here (Fig. 10) of the degeneration of a cluster of endings after severe metrazol treatment, followed by regeneration of a new group of endings with a different pattern. In this case the metrazol treatment caused elimination of an entire terminal arborization. It also caused degeneration of several of the most distal myelin segments of the main fiber as well as some of the corresponding axis cylinder. Nevertheless, regeneration quickly ensued. A new sprout grew out from the main fiber at the place of the original collateral. Its manner of branching, however, bore no resemblance to the pattern of the original arborization. This case, like that of Fig. 9, indicates that the patterns of free unsheathed nerve endings which undergo degeneration are not exactly duplicated by regenerative processes.

Reactions of Nerve Endings in Tadpoles Subjected To Electric Shocks

One of the best means of bringing about alterations in nerve ending patterns is through the use of electric shocks of suitable strength and number. Along with metrazol and insulin treatments for certain human mental diseases, the electric shock therapy has been gaining favor. Observations of nerve ending reactions to electricity in tadpoles, therefore, are of particular interest because of their possible application to nerve ending changes at synapses in the brain of human mental patients following electric shock treatments.

I have subjected tadpoles to various degrees of electrically-induced injury. Excellent case histories have been obtained of nerve fibers and their endings as these suffer injury and then recover. The example given (Fig. 11) shows a swollen nerve fiber on the day following a fairly severe series of electric shocks. One small branch underwent degeneration. Another ending, though somewhat swollen, readily recovered and grew out into new territory.

Other case histories of electric shock injury and recovery indicate that loss of nerve substance may be much greater in extent. In extreme cases an entire collateral may suffer degeneration, as in the metrazol

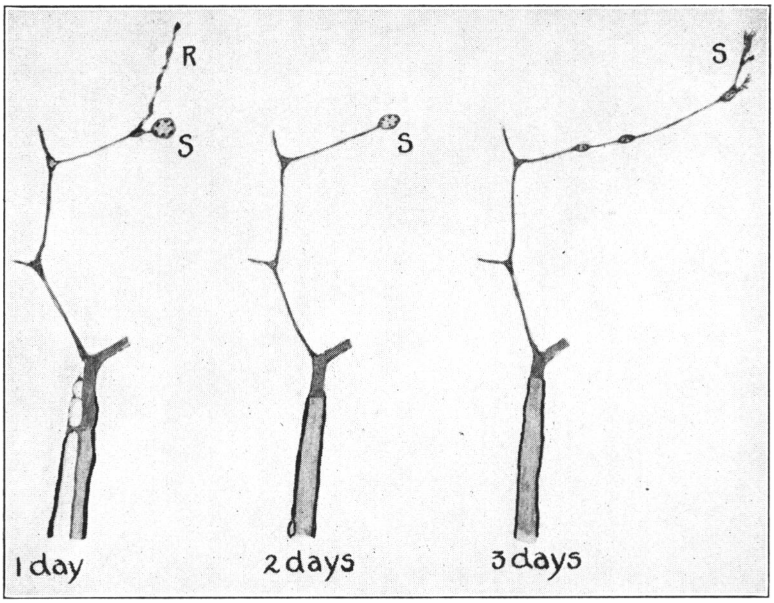

Fig. 11—Elimination of one nerve ending, and swelling and extension of another following electric shock treatments. On the day following a series of electric shocks a myelinated fiber was noted in greatly swollen condition. One ending (S) exhibited swelling. Another short branch (R) was in process of degeneration. On the next day (i.e., two days after the electric shocks) the myelin segment was almost normal; the ending (S) was less swollen; and the ending (R) was gone. On the following day S had advanced markedly and was provided with a growth cone.

case cited above (cf. Fig. 10). In one rather interesting case the electrical treatment was just strong enough to induce degeneration of several myelin segments of a fiber. The enclosed axis cylinder, however, survived although its endings suffered marked reduction with complete elimination of some. During the recovery period of the next few days new branches developed. Further details of electric shock experiments will be published in a separate paper.*

Changes in Nerve Endings of the Distal Stump After Section

After a myelinated fiber is cut typical degenerative changes quickly become discernible in the endings which belong to the distal stump. In the example given (Fig. 12) within the first hour swelling of the endings was noticeable. Retraction, granulation, and liquefaction also took place. Later the branches became exceedingly tenuous and began to fragment.

* This paper has now been published in the *Proc. Am. Philos. Soc.*, 1942, *85*:168.

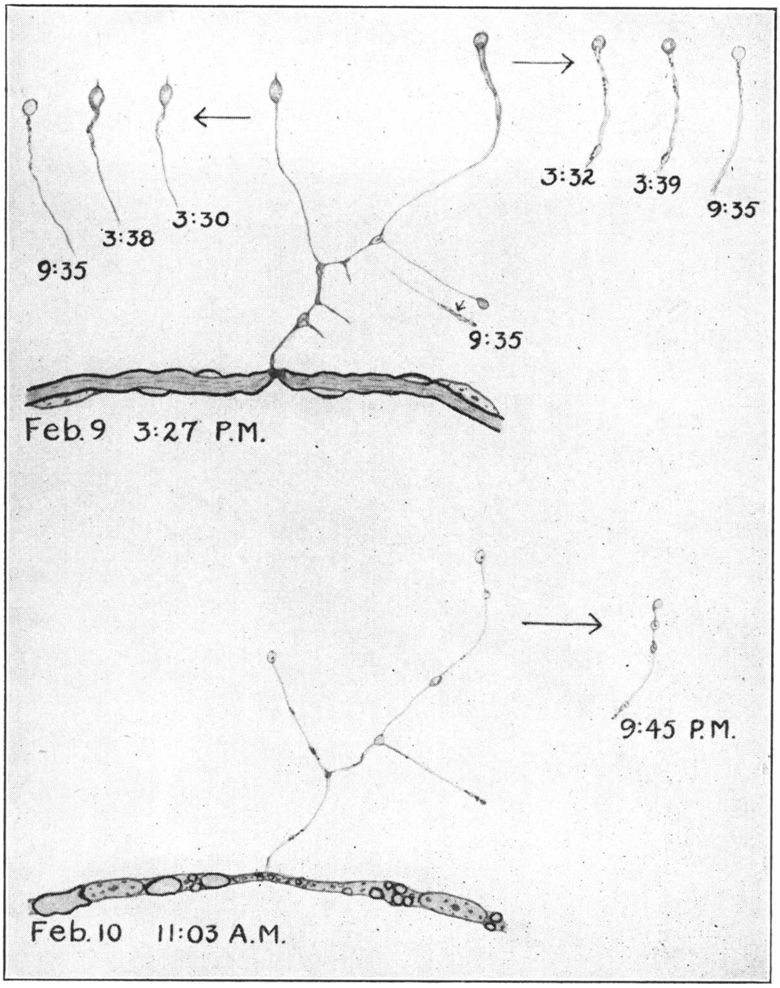

Fig. 12—Degenerative changes in nerve endings after nerve section. On February 9th at 3:03 P.M. a myelinated nerve fiber was cut at a point some distance proximally to the part illustrated. At 3:27 P.M. distinct changes in the nerve endings of the collateral branch were discernible. The ending at the left exhibited swelling and retraction club formation; the ending at the right exhibited swelling and thickening. Other changes became visible during the next six hours at the times indicated. On February 10th at 11:03 A.M. the three endings of the collateral branch were still visible. At this time the collateral was exceedingly tenuous. Myelin degeneration was visible. At 9:45 P.M. the short length of fiber sketched at the right was all that was left of the entire collateral. No trace of this was visible on February 11th.

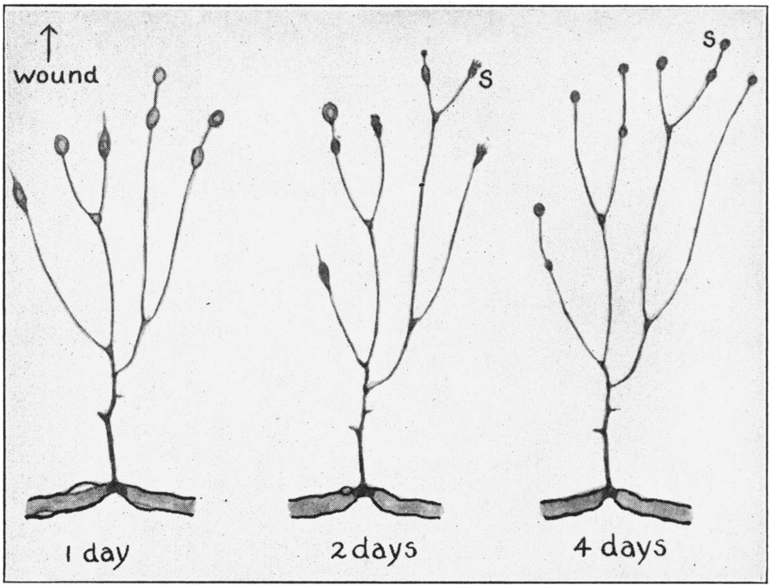

Fig. 13—Response of nerve endings near a wound during subsequent tissue regulation and repair. During the first day after a minor wound infliction a group of nearby nerve endings exhibited swelling and slight retraction. After two days growth cones were present on some of the endings. A new branch (S) was present. One ending, however, still exhibited swelling and one had retracted. After four days several endings had advanced toward the zone in which tissue repairs were taking place. All endings at this time were in the resting state.

It is clear that these degenerative changes in endings belonging to the distal stump bear a decided resemblance to the various irritative changes in endings, such as have been pointed out in many of the experimental treatments described above. In other words, the early changes in an irritated ending which is destined to recover are much like the early changes in a degenerating ending which is destined to be lost completely.

Changes in Nerve Endings Near a Wound As Cellular Regulation and Repair Ensue

If a local wound is made in the tadpole's tail by cutting, bruising, or other means, irritation of nearby nerve fibers takes place. Typical irritative changes of swelling and retraction may be seen readily in favorable cases. If the nerve endings under observation are too close to the wound, or if the wound becomes too extensive, complete degenera-

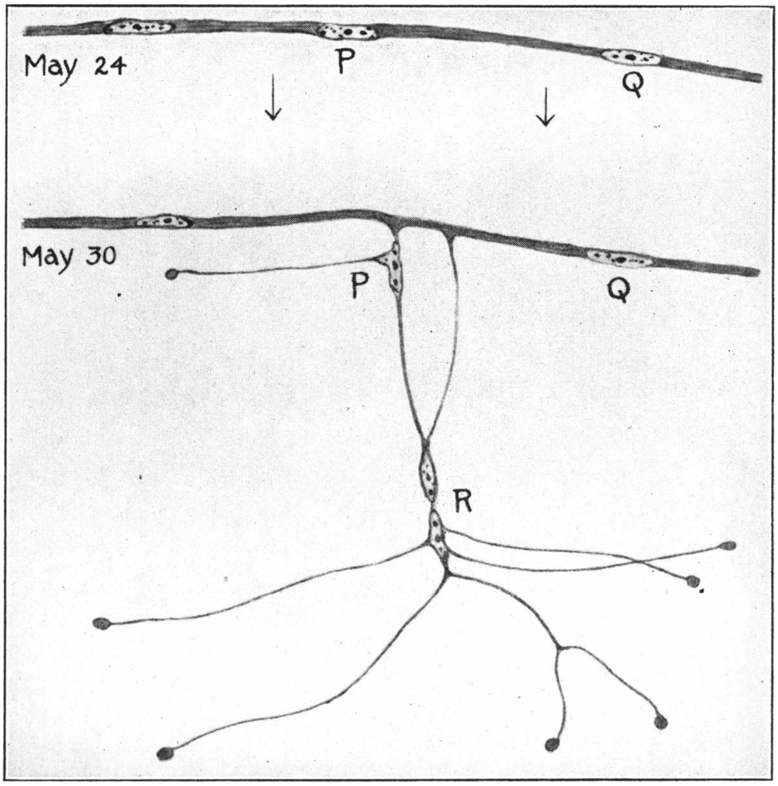

Fig. 14—The origin of collateral sprouts from a nerve following experimental denervation of an adjacent zone. On May 24th by appropriate sectioning of the tadpole tail fin a zone in the direction of the arrows was deprived of its nerve supply. The nerve figured was at the edge of the denervated region. Two new side sprouts arose, one on May 27th and one on May 28th. These grew and by May 30th had given rise to seven endings which supplied a part of the denervated zone. P, Q, and R mark the positions of neurilemma sheath cells. (The two sheath cells at R arose by division from a sheath cell which transferred from a degenerating nerve in the denervated zone.)

tion of endings may ensue. On the other hand, if the wound is too slight or if the endings are too distant, little or no change is visible. In suitable cases, however, excellent case histories of regressive and progressive changes in nerve endings from day to day may be obtained. In the example cited (Fig. 13) the endings of a terminal arborization exhibited definite swelling and retraction during the early period of injury. This was followed by recovery with growth and branching as wound repair proceeded.

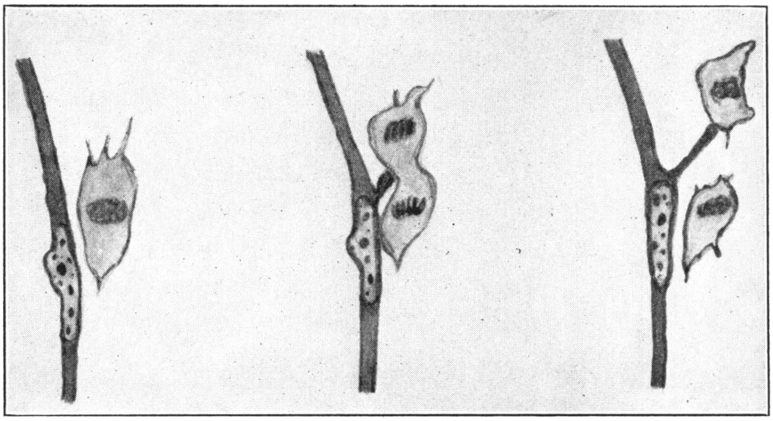

Fig. 15—Origin of a nerve sprout correlated with mitosis of a nearby myoblast. A motion picture record of this case was obtained. A young muscle cell in division was located close to a small unmyelinated nerve and sheath cell. Immediately after the metaphase of mitosis a knob-like bulge appeared at the side of the nerve. This rapidly grew out as a new nerve branch, the tip remaining in contact with one of the daughter cells.

Evocation of Collateral Sprouts from a Nerve Following Experimental Denervation of Adjacent Territory

Obliteration of the nerve supply for a region quickly sets in motion recovery processes that will ultimately lead to the restoration of innervation of the affected region. An important feature of such recovery processes is the response of the nerves nearest the denervated zone. These may give rise to new sprouts which then quickly grow into the denervated territory. Presumably in some manner the denervated zone constitutes a local stimulus with sufficient influence to evoke new sprouts from nearby fibers which otherwise would not have given rise to them. In the example given (Fig. 14) a period of three days was enough to elicit the first new collateral branch from the adjacent nerve.

Nerve Fibers and Endings As Affected by Dividing Cells

Many instances have been recorded in which a nerve fiber exhibited definite changes during the division of a cell in close proximity. In one case (Fig. 15), as a young muscle cell divided by mitosis a new sprout arose from a nearby unmyelinated fiber. The sprout arose at a time of great agitation, namely, immediately after the dividing cell passed

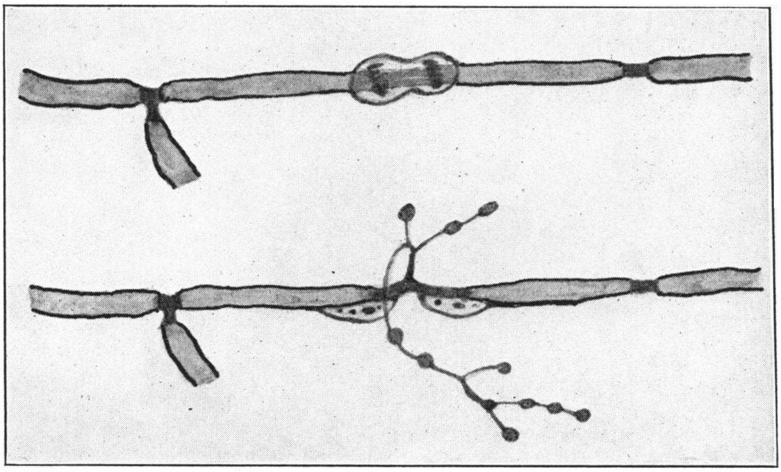

Fig. 16—Origin of a collateral set of endings from a myelinated fiber following sheath cell mitosis. In a young tadpole a sheath cell belonging to the penultimate myelin segment of a fiber underwent mitosis. The myelin became divided into two segments, each with a daughter sheath cell. Three hours after the mitosis a new young cluster of endings was visible, arising from the main fiber at a point between the two daughter sheath cells.

the metaphase stage. At this time the whole locus was in a state of activity, a feature clearly revealed by the fast motion ciné-photomicrographs that were obtained.

A dividing neurilemma sheath cell also constitutes a stimulus for the origin of new collateral nerve sprouts. I have watched several examples, including both myelinated and unmyelinated fibers, in which new branches arose following the metaphase. Motion pictures of dividing neurilemma cells vividly demonstrate that at such a time the nerve fiber swells and becomes more plastic. It is certainly stimulated mechanically, and possibly chemically, by the dividing cell. The case selected here for illustration (Fig. 16) shows the origin of a new collateral branch after mitosis of a neurilemma cell associated with a myelin segment.

Neurilemma cells also play a part in aiding regenerating nerve fiber tips to get past obstacles. Such aid is sometimes accompanied by mitotic division of the neurilemma cell. Examples of this have been presented in previous papers.[3,a,b,d]

Connective tissue cells in division may also cause activity of nerve endings provided they are in close juxtaposition. In two cases I have

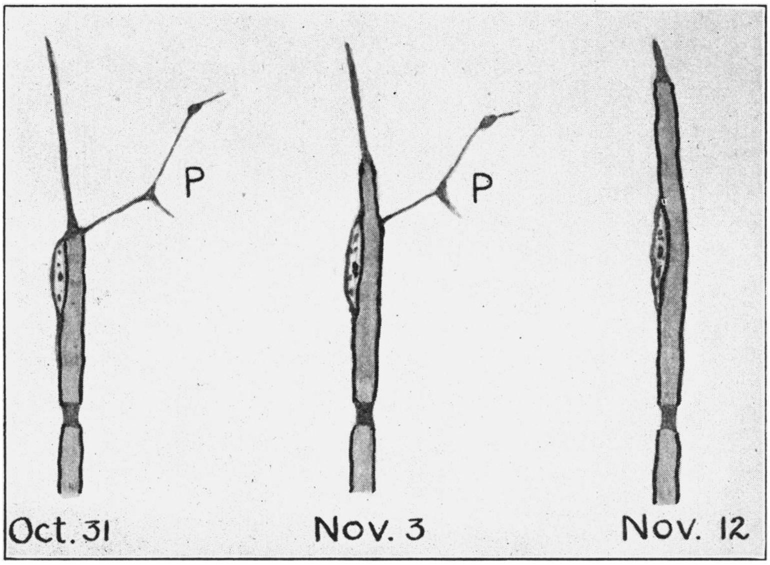

Fig. 17—Elimination of a collateral branch by advancing myelination. On October 31st a side branch (P) was present at the end of a terminal myelin segment. During the next three days the myelin advanced beyond the side branch, as indicated. By November 12th the side branch had become entirely eliminated.

seen a nerve ending of resting type become transformed into one of growing type, as a result of the motion of a contiguous dividing connective tissue cell. In one case the nerve ending tip advanced a short distance. In the other case there was no advance; after a brief period of activity characterized by slow movements of delicate pseudopods, the nerve tip again became transformed into the resting type with spherical end bulb.

Myelination as a Factor in Elimination of Nerve Endings

Myelination accompanies the growth and maturing of nerve fibers. One feature of this is the increase in the size and complexity of many of the terminal arborizations. Nevertheless, another important aspect of myelin sheath formation is the complete elimination of some collateral branches with their endings. I have observed many cases of this. In the example given (Fig. 17), a collateral branch was eliminated during growth of a myelin segment. In a previous paper[3,b] I have illustrated a similar elimination of a much larger collateral side branch which had itself become ensheathed with a myelin segment.

The myelination process, however, does not always eliminate collateral branches which are not located at nodes of Ranvier. I have followed the histories of many such internodal collaterals and noted their survival up to the time at which metamorphosis took place.

Miscellaneous Observations

A few other observations of nerve ending adjustments may be mentioned briefly. Typical regressive changes in nerve fibers and their endings have been induced by heat (37°-40° C.) and cold, by relatively weak hypertonic sodium chloride solutions, by X-rays, by insulin extracts, and by foul aquarium water in which putrefaction is occurring. Recovery takes place after such irritants provided the injury is not too great.

Circulatory disorder is usually associated with nerve irritation. Chloretone, alcohol, metrazol, insulin, electric shocks, and hypertonic salt solutions all cause a slowing of the circulating blood. Nerve ending changes follow. Rarely, as in one case of chloretone treatment, an advancing growth cone of a regenerating fiber continued its activity and advanced an appreciable distance after the circulation in the entire tail had ceased.

As the time for metamorphosis of the frog tadpole approaches, the tail fin suffers increasing reduction in size. Irritative and degenerative changes become apparent in all of the tissues. Nerve fibers and their endings become affected. The nerve endings exhibit swelling and may suffer some retraction. Occasionally, however, growth and extension of an ending is visible even in a markedly reduced tail fin.

Connective tissue cells often block advancing nerve endings. Prolonged blocking may lead to nerve autotomy. Blocking of a rapidly regenerating nerve fiber tip may be followed by retraction and growth in a new direction, or by retraction and branching with growth of the branches in new directions past the obstruction.

Ciné-photomicrographs of Nerve Ending Adjustments

Ciné-photomicrographs of both normal and fast motion types have been obtained directly from living tadpoles. These record the typical changes in nerve endings under ordinary conditions and under conditions of irritation, injury, and recovery. Fast motion ciné-photomicrographs are particularly valuable in revealing slow tissue changes. Prolonged day-to-day case histories are suitably shown by pictures taken at the normal rate. The motion pictures exhibited include, in addition to changes in nerve fibers and nerve endings, the activities of some other cells which are associated with nerve ending adjustments.

The subjects of some of the pictures follow.

1. Rapidly growing nerve tip blocked temporarily by a connective tissue cell process.

2. Rapidly growing nerve tip advancing through the tissues after passing a blockade formed by two connective tissue cells.

3. Two growth cones diverging as they advance.

4. Retraction of a growth cone (two examples).

5. Retraction of an actively advancing nerve tip induced by alcohol treatment, followed by resumption of growth along a new route with the restoration of normal conditions.

6. Reactions of the myelin sheath, axis cylinder, and nerve endings during and following alcohol treatments.

7. Swelling, retraction, recovery, autotomy, and degeneration of nerve endings in alcoholized tadpoles.

8. Degeneration of an entire cluster of nerve endings caused by severe metrazol-induced injury, followed by regeneration of a new nerve ending pattern at the former site.

9. Epithelial cells (overlying irritated cutaneous nerve endings) displaying rapid movements of readjustment, three hours after metrazol treatment.

10. Lymphocyte movements within the neurilemma of an adjusting nerve trunk after metrazol injury.

11. Continuous, though slow, advance of a cutaneous ending over a four day period in a tadpole nearing metamorphosis.

12. The retraction of an ending, initiated by starvation, continuing even after normal food conditions have been restored.

13. The genesis of a nerve branch in close proximity to, and probably stimulated by, a dividing myoblast.

14. Nerve adjustments associated with division and movements of sheath cells.

15. Growth of fibroblast process following fibroblast mitosis (for comparison with nerve tip growth).

16. Markedly swollen nerve endings on the day following severe

electric shock treatments.

17. Electrical injury of nerve endings with swelling and degeneration, followed during the next four days by nerve ending extension and establishment of new connections.

18. Clotting at the muscle-tendon junction of a fiber of striated muscle during electrical injury.

19. Two case histories showing successive retraction and advance of the same individual nerve endings, correlated with alternating periods of starvation and good nutrition.

20. Chronic swelling and autotomy of an end bulb of a fiber in a starved tadpole, followed by development of an abortive growth cone tip.

21. Comparison of macrophage activity during ingestion of a swollen degenerating nerve ending and during ingestion of an extravasated red blood cell.

Discussion

A few points suggested by the foregoing observations may be discussed briefly. It is clear that while there are many varieties of irritation and injury, nerve fiber endings display only one general pattern of changes. This includes swelling, retraction, and variable amounts of degeneration. Likewise, the changes during recovery follow one general pattern. This includes reduction of swelling, growth and branching of endings, and the establishment of new terminal positions.* The point has already been made that the end arborizations are free and unsheathed. There is, therefore, no duplication of an original pattern of nerve endings as a part of the recovery process following a period of regressive change. This feature makes for flexibility. Nerve endings are not absolutely fixed and stable. They adjust themselves to changing stresses and strains.

It should be emphasized that my observations deal only with free unsheathed endings, peripherally located. This type, however, is present in enormous numbers in the central nervous system, taking part in the synapses between nerve cells. Cajal[1,4] has given us many illus-

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* Nerve endings represent the part of the nerve cell that is most distant from the cell nucleus. In general, it is true of cells having long processes that the most peripheral parts suffer regressive change first. I have watched irritated connective tissue cells, pigment cells, and the endothelial cells of blood and lymph capillary sprouts. During the phases of irritation and recovery, the tips of the processes of these cells exhibit changes much like those of nerve endings. The behavior of the nerve endings, therefore, is not unique.

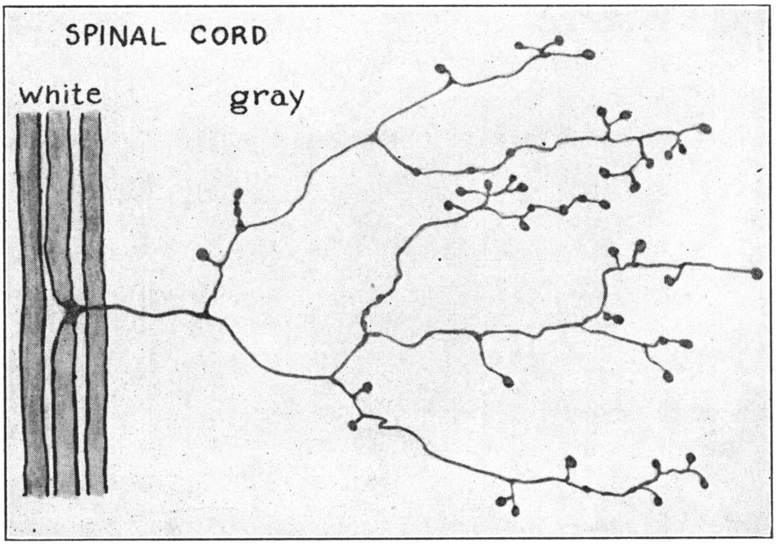

Fig. 18—A terminal arborization of nerve endings in the gray matter of the cat's spinal cord, arising from a collateral branch of a myelinated fiber in the white matter. Redrawn from Cajal, representing two illustrations combined. This illustration of synaptic nerve endings in the central nervous system is strikingly similar to that of peripheral nerve endings at the skin (cf. Fig. 1).

trations. In the example reproduced here from the cat's spinal cord (Fig. 18) is shown a collateral branch with its terminal arborization in the gray matter. This tree of free nerve endings is quite like those in the frog tadpole which have formed the subject of my investigations. Similar end arborizations are present also in the brain.

Unfortunately direct observations cannot be made on nerve endings located within the central nervous system. Case histories of individual endings, therefore, have never been recorded. The fixed preparations of Cajal,[1] however, clearly show that a centrally located nerve ending does not always have a resting end bulb tip. It may have a swollen irritated tip, a growth cone tip, or a retraction club tip. Endings exhibiting degeneration and autotomy are also apparent.

Accordingly, there is little doubt in my mind but that the free nerve endings of the central nervous system may undergo changes during irritation, injury, and recovery much like those of peripherally located endings. This is significant because of the role such endings play at synapses. The conclusion seems justified that some flexibility in synaptic connections is possible; that certain changes may take place from time to time with the elimination of some old synapses and the establishment of some new ones.* I have already proposed such an anatomical mechanism as a basis to account for the changed mental outlook that sometimes occurs in metrazol-treated human beings.[3,f]

Not all synapses are alike. In some cases there is extensive contact between the two nerve cells concerned through complex interlacing of their processes. In other cases there is relatively limited contact, the endings of one nerve cell merely touching at one or more places the endings, or processes, or cell body of the other nerve cell.** The former type of synapse would probably be more stable than the latter type.

Nerve fibers which supply striated muscle are usually ensheathed with neurilemma all the way to the muscle fiber end plate.[7] Likewise, nerve fibers which end in special sensory corpuscles of the encapsulated type are usually ensheathed with neurilemma all the way to the corpuscle.[7] Such endings would also be more stable than those of the free type. If injury did take place the surviving neurilemma would function to direct the regenerating fiber along the original path to the end structure concerned.

In conclusion, it seems probable to me that nerve ending changes accompany many bodily disorders of both acute and chronic types; such, for example, as during severe infectious diseases characterized by toxins and fever, during states of marked hormonal imbalance, during chronic vitamin deficiency, during marked circulatory and kidney disturbances, and during the gradual tissue degeneration that characterizes the approach of senility. In man, the synaptic changes in the brain probably would be of most significance in accounting for whatever mental changes might accompany such general bodily disorders.

Summary

1. The nerve endings of terminal arborizations of myelinated fibers in frog tadpoles, though relatively stable, may undergo adjustments under both normal and experimental conditions. The principal changes exhibited are swelling, retraction, extension, branching, autotomy, and

* Extensive regeneration of nerve cells and fibers does not occur in the brain and spinal cord, as is well known. Nevertheless, Cajal[1] presents many illustrations which lead me to the conclusion that the delicate branches of terminal arborizations readily adjust themselves either progressively or regressively.

** I agree with the "contact idea" as to the nature of the synapse. Strong evidence for this has been presented recently by Bartelmez and Hoerr[5] and by Bodian.[6]

degeneration. Essentially similar nerve ending adjustments result from slow chronic changes and from rapid acute changes.

2. During a period of general bodily growth, a terminal arborization increases in the size and number of its branches. From time to time individual endings may become swollen. They then may extend by growth cone activity, or retract, or suffer degeneration. Sometimes autotomy of a branch, or a part of a branch takes place. Some endings of cutaneous fibers are aberrant and grow in a deep, instead of a superficial, direction. Such branches become eliminated by retraction, or degeneration, or as further growth takes place they may change their course in such manner as to extend in a superficial direction.

3. Starvation induces regresssive changes of swelling, retraction, and degeneration. Successive retraction and growth of the same individual endings may be induced experimentally by imposing on tadpoles alternating periods of starvation and good nutrition.

4. Treatments of tadpoles with alcohol or with chloretone may cause typical regressive changes in nerve endings. With such treatments alternating with recovery periods, regressive and progressive changes may be induced successively in individual nerve endings. The growth cone tips of rapidly regenerating nerve fibers are particularly susceptible to experimental modifications of this sort.

5. At rest the end bulb (bouton) at the tip of a branch is in the gel state. During irritation it undergoes increasing change toward the sol state as swelling ensues. During recovery it returns to the gel state. Retracting and growing tips are in an intermediate state.

6. During regeneration after injury, nerve fibers and their branches duplicate an original pattern only as far as they are ensheathed by the original neurilemma. Free unsheathed nerve endings, therefore, establish new connections during recovery.

7. Nerve fibers and their endings display marked changes of irritation and injury in tadpoles subjected to metrazol, or to insulin, or to electric shocks. With recovery after each of these treatments the patterns of individual nerve ending clusters may be quite different from the patterns before treatment.

8. Nerve ending changes are also apparent after treatments with hypertonic sodium chloride solutions and after exposure to moderate rise in temperature.

9. The changes in irritated or slightly injured nerve endings which

will recover resemble closely the early changes in nerve endings undergoing trophic degeneration which will not recover.

10. Local wounds sometimes stimulate adjustments of nearby nerve endings in uninjured territory. Swelling, retraction, extension, and branching of endings may result.

11. A denervated zone may evoke new side sprouts from nearby uninjured nerve fibers. Collateral innervation may thus be established.

12. New nerve sprouts sometimes appear on fibers that are contiguous to dividing cells (neurilemma cells, myoblasts, and fibroblasts). This takes place after the metaphase, during a time of marked agitation.

13. The advance of the myelin sheath on a fiber is a factor in causing elimination of some branches by retraction or autotomy. Even large branches with myelin segments are occasionally eliminated.

14. Free nerve endings which are present in large numbers in the central nervous system probably undergo adjustments similar to those of free cutaneous endings. Synaptic changes would be possible, therefore, from time to time. Thus, changes in brain synapse patterns might be brought about as a result of shock treatments, severe intoxications and fevers, and marked nutritional or hormonal imbalance. Such changes also probably accompany ordinary growth and maturation of the brain.

REFERENCES

1. Cajal, S. Ramon y. *Degeneration and regeneration of the nervous system.* London, Oxford Univ. Press, 1928.
2. Harrison, R. G. The outgrowth of the nerve fiber as a mode of protoplasmic movement, *J. Exper. Zoöl.,* 1910, *9:*787; and The reaction of embryonic cells to solid structures, *J. Exper. Zoöl.,* 1914, *17:*521.
3. (a) Speidel, C. C. Studies of living nerves; movements of individual sheath cells and nerve sprouts correlated with process of myelin-sheath formation, *J. Exper. Zoöl.,* 1932, *61:*279; (b) Studies of living nerves, activities of ameboid growth cones, sheath cells and myelin segments, *Am. J. Anat.,* 1933, *52:*1; (c) Studies of living nerves; growth, regeneration, and myelination of peripheral nerves in salamanders, *Biol. Bull.,* 1935, *68:*140; (d) Studies of living nerves; phenomena of nerve irritation and recovery, degeneration and repair, *J. Comp. Neurol.,* 1935, *61:*1; (e) Studies of living nerves; alcoholic neuritis and recovery, *J. Comp. Neurol.,* 1936, *64:*77; and (f) Studies of living nerves; effects of metrazol on tissues of frog tadpoles, with special reference to the injury and recovery of individual nerve fibers, *Proc. Am. Philos. Soc.,* 1940, *83:*349.
4. Cajal, S. Ramon y. *Histology.* Baltimore, Wood, 1933.
5. Bartelmez, G. W. and Hoerr, N. L. Vestibular club endings in Ameiurus; further evidence on morphology of synapse, *J. Comp. Neurol.,* 1933, *57:*401.
6. Bodian, D. Structure of the vertebrate synapse, *J. Comp. Neurol.,* 1937, *68:*117.
7. Boeke, J. Nerve endings, motor and sensory, in *Cytology and cellular pathology of the nervous system,* ed. by W. Penfield, New York, Hoeber, 1932, v. *1,* sect. 6.